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# THE UNITED STATES OF AMERICA

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United States Patent and Trademark Office

*February 23, 2005*

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**APPLICATION NUMBER: 60/577,790**

**FILING DATE: *June 08, 2004***

**RELATED PCT APPLICATION NUMBER: *PCT/US05/03369***



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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

**EV440868342 US****INVENTOR(S)**

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Rolf Ulrich	Halden	Baltimore, MD
David Robert	Colquhoun	Westminster, MD

Additional inventors are being named on the \_\_\_\_\_ separately numbered sheets attached hereto

**TITLE OF THE INVENTION (500 characters max)****Method for detection of Schistosome Parasites**

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OR

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**ENCLOSED APPLICATION PARTS (check all that apply)**☒ Specification Number of Pages: 17

CD(s), Number \_\_\_\_\_

☐ Drawing(s) Number of Sheets \_\_\_\_\_

Other (specify) \_\_\_\_\_

☐ Application Data Sheet. See 37 CFR 1.76**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**☒ Applicant claims small entity status. See 37 CFR 1.27.☐ A check or money order is enclosed to cover the filing fees.☐ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: \_\_\_\_\_☒ Payment by credit card. Form PTO-2038 is attached.FILING FEE  
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are: \_\_\_\_\_

[Page 1 of 2]

Respectfully submitted,

SIGNATURE

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TYPED or PRINTED NAME

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Date

6/8/04

REGISTRATION NO.

55,601

(if appropriate)

Docket Number:

4473**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Cheryl Rydman

Signature

**U.S. Provisional Patent Application**

**JHU Ref. No. DM-4473**

# **Method for the Detection of Schistosome Parasites**

**Inventors:**

**Rolf Halden**

**David Colquhoun**

All publications, patents and patent applications disclosed herein are incorporated into this application by reference in their entirety.

For example: "Sambrook et al, Molecular Cloning, A Laboratory Manual (volumes I-III) 1989, Cold Spring Harbor Laboratory Press, USA" and "Harlowe and Lane, Antibodies a Laboratory Manual 1988 and 1998, Cold Spring Harbor Laboratory Press, USA" provide sections describing methodology for antibody generation and purification, diagnostic platforms, cloning procedures, etc. that may be used in the practice of the instant invention.

The following claim(s) of this provisional application are not to be construed as limiting the disclosed invention(s). The claim(s) are included for compliance with patent application structural regulations that may be imposed by international patent offices.

We claim:

1. A method of identifying parasites in a sample, comprising:
  - harvesting specimens from said sample;
  - extracting proteins from said specimens;
  - generating peptides from said proteins;
  - performing peptide fingerprint analysis of said peptides; and,
  - analyzing said fingerprint for the presence of parasite biomarkers.

## Report of Invention Disclosure Form (ROI)

This form is to be completed and submitted to the JHU office of Licensing and Technology Development (LTD) by anyone who believes they have developed a new invention. The purpose of this form is to enable LTD to evaluate whether legal protection to the invention will be sought and/or commercialization pursued. Please submit this form with all inventor(s) and Department Director(s) signatures. Visit the LTD web site at <http://jhu.edu/technology/roi.html> for HTML and Word downloadable formats of this form.

### INVENTION INFORMATION

**Title of Invention:** Method for the Detection of Schistosome Parasites

<b>Name of Lead Inventor:</b>	Halden	Rolf	Ulrich
	Last	First	Middle
			Degree

**Lead Inventor Information:** [The Lead Inventor is the primary contact person for LTD on all matters associated with this Report of Invention, including processing, patent prosecution and licensing. For reasons of administrative efficiency, it is the responsibility of the Lead Inventor to keep all other JHU inventors named on this Report of Invention informed of the status of such matters.]

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**Citizenship:** German

Are you a Howard Hughes Medical Institute employee or investigator?      ☐ Yes ☒ No

Are you a Kennedy Krieger Institute employee or investigator?      ☐ Yes ☒ No

**Additional inventors:** ☒ Yes ☐ No      If yes, please complete Additional Inventors section for each inventor.

**LTD Internal Use Only:** REF- 4473      TLA GHS      Field of Use 20

**ADDITIONAL INVENTION INFORMATION**  
Please copy this page for additional inventors as necessary

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Are you a Howard Hughes Medical Institute employee or investigator? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No Are you a Kennedy Krieger Institute employee or investigator? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No				

## INVENTION DESCRIPTION

Describe the invention completely, using the outline given below. Please provide an **electronic copy** of the invention disclosure document, references, and abstracts in Windows format on CD-ROM or floppy disk if possible

- 1. Marketing Summary** [Please provide a non-confidential summary of the invention that can be used for marketing purposes. Unique details that are published may also be included.]

**Brief Description:**

Schistosomes cause serious disease by living parasitically in the blood vessels draining the gut and bladder of humans and warm-blooded animals where they produce eggs that are passed out in the urine and feces. In man and animal hosts (dogs, cats, and livestock), infection produces severe debilitation often resulting in cirrhosis, bladder cancer and death. The disease (a) ranks higher in prevalence than HIV/AIDS, (b) is a leading cause of cirrhosis and bladder cancer in the world, and (c) is second only to malaria in importance in endemic areas. Schistosome cercariae are water-borne parasitic larval forms that orientate to the water surface where they wait to contact and invade vertebrate hosts.

The novel monitoring method disclosed here allows for the selective and specific detection of Schistosome cercariae in environmental and biological samples. It informs at once on (a) the presence of Schistosome parasites, (b) the specific lifecycle stage of the parasite present, and (c) the infectivity of the pathogen. The method is suitable for large volume, high-throughput analysis of both environmental and biological samples. As such, it addresses an important niche in the worldwide detection, control and management of Schistosome diseases including Bilharzia and Swimmer's Itch.

**Potential Commercial Use:**

The Schistosome monitoring technique could be sold as a license, product and/or service. The technology can be used to obtain in a one-step process information on the presence, lifecycle stage, and infectivity of Schistosome parasites. The technology invites application in the following areas: environmental monitoring of Schistosome parasites, medical screening of biological specimens for Schistosome parasites, risk assessment and exposure assessment, protection of health of civilians and military personnel deployed in the United States and abroad, prevention of bioterrorism activities employing modified Schistosome parasites as infectious vehicles.

**Marketing Goal:**

Johns Hopkins University is seeking licensees for this technology.

**Keywords:**

Parasite detection, tropical disease, water-borne disease, Schistosomiasis, Bilharzia, Swimmer's Itch.

**SOFTWARE** – Does this disclosure include a software element or software is implemented in the invention

☐ Yes ☒ No

If yes, please complete the Software Information Form which can be found at:

**BIOLOGICAL MATERIAL** – Does this disclosure include biological material.

☐ Yes ☒ No

If yes, please attach a list of materials for reference. A Tangible Property Report of Invention form may be completed if the disclosure is biological materials only. You can find this form at: <http://www.hopkinsmedicine.org/lbd/otl/>

- 2. Problem Solved** [Describe the problem solved by this invention]

A detection technique is being disclosed, and has been reduced to practice, allowing for the determination of the presence, lifecycle stage, and infectivity of Schistosome parasites in environmental and biological samples.

**8. Workable Extent/Scope** [Describe the future course of related work, and possible variations of the present invention in terms of the broadest scope expected to be operable; if a **compound**, describe substitutions, breadth of substituents, derivatives, salts etc., if **DNA or other biological material**, describe modifications that are expected to be operable, if a **machine or device**, describe operational parameters of the device or a component thereof, including alternative structures for performing the various functions of the machine or device]

The disclosed method has a broad workable extent. The detection technique may be applied not only for environmental monitoring but also for the medical analysis of biological specimens including, but not limited to, blood, urine, and feces. The detection strategy by MALDI-TOF MS may be replaced by less capital-intensive, lower cost alternatives including, but not limited to, the use of affinity materials for lifecycle-specific proteins whose binding to the sensor is read by optical methods. Large volume high-throughput analysis of environmental and biological samples may be accomplished using "protein affinity arrays" and "protein chips" containing antibodies and docking sites for the proteinaceous targets described here. The method may also be integrated in a monitoring device allowing for environmental sampling and analysis in the field.

**9. References** [Please cite relevant journal citations, patents, general knowledge or other public information related to the invention and distinguish between references that (A) contain a description of the current invention from those that (B) contains background information.]

**A**

None.

**B**

World Health Organization (1998). Report of the WHO Informal Consultation on Schistosomiasis Control. WHO/CDS/CPC/SIP/99.2. Geneva, Switzerland. ([http://www.who.int/entity/wormcontrol/documents/publications/en/99\\_2en.pdf](http://www.who.int/entity/wormcontrol/documents/publications/en/99_2en.pdf))

Ross, A. G., Sleight, A. C., LI, Y., Davis, G. M., Williams, G. M., Jiang, Z., Feng, Z., and D. P. McManus (2001). Schistosomiasis in the People's Republic of China: Prospects and Challenges for the 21<sup>st</sup> Century. *Clinical Microbiol. Rev.* 14(2):270-295.

Valle, C., Festucci, A., Calogero, A., Macri, P., Mecozzi, B., Liberti, P., and D. Cioli§ (1999). Stage-specific Expression of a *Schistosoma mansoni* Polypeptide Similar to the Vertebrate Regulatory Protein Stathmin\*. *J. Biol. Chem.* 274(48):33869-33874.

**3. Novelty** [Identify those elements of the invention that are new when compared to the current state of the art]

Existing techniques for the detection of Schistosome parasites are labor-, time-, and cost-intensive, as they rely on the use of microscopic observation by trained parasitologists. The here presented novel technique overcomes these limitations by furnishing a rapid, selective and highly specific means of monitoring for the presence, lifecycle stage and infectivity of Schistosome parasites. The assay is unbiased and suitable for automated high-throughput analysis of both environmental and biological samples.

**4. Potential Commercial Use** – [What products can be produced with this invention.]

**Potential Commercial Use:**

The Schistosome monitoring technique could be sold as a license, product and/or service. The technology can be used to obtain in a one-step process information on the presence, lifecycle stage, and infectivity of Schistosome parasites. The technology invites application in the following areas: environmental monitoring of Schistosome parasites, medical screening of biological specimens for Schistosome parasites, risk assessment and exposure assessment, protection of health of civilians and military personnel deployed in the United States and abroad, prevention of bioterrorism activities employing modified Schistosome parasites as infectious vehicles.

**Keywords – Please circle the categories and keywords that accurately describe the present invention.**

**CHEMICAL**

- ☐ Additives
- ☐ Alternative Energy
- ☐ Antioxidants
- ☐ Batteries
- ☐ Catalyst
- ☐ Coal Conversion
- ☐ Coatings
- ☐ Effluent Treatment
- ☐ Elastimers
- ☐ Electrochemistry
- ☐ Exhaust Treatment
- ☐ Foams
- ☐ Food Chemistry
- ☐ Fuel Cells
- ☐ Gas Conversion
- ☐ Gels
- ☐ Monomers
- ☐ Oxidation
- ☐ Petroleum
- ☐ Photochemistry
- ☐ Polymers
- ☐ Remediation
- ☐ Solvents

**DIAGNOSTIC**

- ☐ Antibody
- ☒ Assay
- ☐ Biochip
- ☐ Contrast Agent
- ☒ Detection
- ☐ DNA Probe
- ☐ Elisa
- ☐ Imaging
- ☐ Immunoassay
- ☐ In Situ
- ☒ Marker
- ☐ Measurement
- ☐ MRI
- ☐ Point of Use
- ☐ Radioisotope
- ☐ Transgenic
- ☐ Ultrasound

**GENOMICS**

- ☐ Allele
- ☒ Bioinformatic
- ☐ cDNA
- ☐ Epidemiology
- ☐ EST
- ☐ Gene
- ☐ Homologue
- ☐ Isogene
- ☐ Library
- ☐ Mutation
- ☐ Pharmacogenomics
- ☐ Polymorphism
- ☐ Positional Cloning
- ☒ Proteomics
- ☐ Receptor
- ☐ RNA
- ☐ Target Validation

**MEDICAL DEVICE**

- ☐ Delivery
- ☒ Diagnosis
- ☐ Imaging
- ☒ Measurement
- ☐ Optical
- ☐ Safety
- ☐ Surgical
- ☐ Treatment

**RESEARCH TOOL**

- ☐ Animal Model
- ☐ Antibody
- ☐ Cell Line
- ☐ Culture
- ☐ Directed Evolution
- ☐ DNA Probe
- ☐ DNA/RNA Sequencing
- ☐ DNA/RNA Synthesis
- ☐ Electrophoresis
- ☐ Elisa
- ☐ Enzyme
- ☐ Equipment
- ☐ Expression System

- ☐ Immunoassay
- ☐ Label
- ☐ PCR
- ☐ Protein Sequencing
- ☐ Protein Synthesis
- ☐ Reagent
- ☐ Spectroscopy
- ☐ Tissue Culture
- ☐ Vector

**SCREENING**

- ☒ Assay
- ☐ Biochip
- ☐ Combinatorial Biology
- ☐ Combinatorial Chemistry
- ☒ Detection
- ☐ HTS
- ☐ Phage Display
- ☐ Screen
- ☐ Target

**THERAPEUTIC**

- ☐ Analgesic
- ☐ Anesthetic
- ☐ Angiogenesis
- ☐ Antibiotic
- ☐ Antibody
- ☐ Antifungal
- ☐ Antiinflammatory
- ☐ Antisense
- ☐ Antiviral
- ☐ Apoptosis
- ☐ Cell Signaling
- ☐ Cell Therapy
- ☐ Disease Model
- ☐ Drug Delivery
- ☐ Drug Design
- ☐ Fertility
- ☐ Gene Therapy
- ☐ Hormone
- ☐ Immunotherapy
- ☐ Natural Product
- ☐ Peptides

- ☐ Pro-drug
- ☐ Proteins
- ☐ Small Molecule
- ☐ Tissue Engineering
- ☐ Transplant
- ☐ Vaccine
- ☐ Virus
- ☐ Wound Healing

**DISEASES**

- ☐ Aging
- ☒ Blood
- ☒ Cancer
- ☐ Cardiovascular
- ☒ Dermatologic
- ☐ Endocrine
- ☒ Gastrointestinal
- ☒ Genitourinary
- ☐ Hepatic
- ☐ Immune
- ☒ Infectious
- ☐ Metabolic
- ☐ Musculoskeletal
- ☐ Neurological
- ☐ ObGyn
- ☐ Ophthalmological
- ☐ Oral
- ☐ Pediatric
- ☐ Psychiatric
- ☐ Respiratory

**ADDITIONAL KEY WORDS:**

**STAGE OF DEVELOPMENT**

- ☒ Unspecified
- ☐ Discovery
- ☐ Preclinical
- ☐ Prototype
- ☐ Phase I
- ☐ Phase II
- ☐ Phase III
- ☐ NCE

**7. Detailed Description of the invention** - On a separate page(s), attach a detailed description of how to make and use the invention. The description must contain sufficient detail so that one skilled in the same discipline could reproduce the invention. Include the following as necessary:

- 1- data pertaining to the invention;
- 2- drawings or photographs illustrating the invention;
- 3- structural formulae if a chemical;
- 4- procedural steps if a process;
- 5- a description of any prototype or working model;

In general, a manuscript that has been prepared for submission to a journal will satisfy this requirement.

See attached pages.

# **Detection of Proteinaceous Biomarkers Revealing the Presence, Lifecycle Stage, and Potential Infectivity of Schistosome Parasites Using Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)**

Rolf U. Halden and David Colquhoun

## **Abstract**

Human infections with parasitic Schistosomes represent the leading cause of cirrhosis and bladder cancer in the world. At present, the worldwide control and prevention of schistosomiasis is hampered by a lack of rapid, selective and sensitive techniques allowing for the automated high-throughput analysis of Schistosome parasites in environmental and biological samples. Here we describe the use of matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the detection of proteinaceous biomarkers reporting on the presence, lifecycle stage, and potential infectivity of Schistosome parasites in aqueous samples. The assay presents a novel technique for detecting Schistosome parasites. Its application to environmental and biological specimens may promote control and prevention of Schistosomiasis, a disease ranking higher in prevalence than HIV/AIDS.

## **Introduction**

Schistosomes are parasites in a wide variety of warm-blooded hosts (Ross et al. 2001). In mammals and humans, they cause serious disease by living in the blood vessels draining the gut or bladder where they reproduce and produce eggs that are passed out in the urine and feces. In aquatic birds, they also parasitize the intestinal blood vessels and undertake a similar life pattern with eggs of the parasite being passed out in the droppings of the infected bird. In both these instances, the eggs hatch when passed into fresh water. The liberated free-swimming larvae search for appropriate species of freshwater snails and proceed to invade the tissue and begin asexual reproduction. The products of this reproduction are a second type of larva, the cercaria. Cercariae are free-swimming organisms that orientate toward the air-water interface to penetrate the skin of the next host.

When the cercariae of bird schistosomes attack a person, the invading parasite does not mature, but produces an immunological hypersensitive reaction in the skin, known as Swimmer's Itch. This local inflammation and severe irritation of the skin occurs where the parasite penetrated the skin. Although no serious infection results, the condition can be debilitating and disfiguring when large numbers of bird cercariae are involved. Swimmer's Itch is a severe problem affecting recreational swimmers in the American Great Lakes and in many parts of the world where the appropriate species of freshwater snails occur.

Human schistosomiasis occurs in most of Africa, West Asia, parts of the Caribbean and South America, and in China and South East Asia. Hundreds of millions of people are affected and the results of infection produce severe debilitation and even

death. It ranks higher in prevalence than HIV/AIDS and second only to malaria in importance in endemic areas. As people are repeatedly infected during their lives, chronic infection frequently leads to severe effects in the adult population as well as in children.

Common to these two parasitic problems is the method of transmission via certain species of freshwater snails and parasite larvae (cercariae) that emerge in vast numbers from infected snails to attack warm-blooded host animals and humans. Cercariae are released from infected snails mostly during the summer months following exposure to sunlight. Their release is adapted to the life patterns of their hosts and dependent on the presence of snail populations serving as parasitic hosts. Research has shown that infection is often focal, and emission of cercariae is diurnal.

The active concentration of cercariae at the air-water interface presents an opportunity for detecting the parasite. Until now, detection of the parasite required skimming off of the cercariae and time-consuming and labor-intensive visual observation by a trained parasitologist. Here we describe an alternative approach for the detection of Schistosome parasites suitable for revealing in a one-step process the presence of the pathogen at the species level, lifecycle stage, and potential infectivity. The method makes use of matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) targeting proteinaceous biomarkers that are lifecycle and species specific, and expressed only by infectious forms of the pathogen.

## Materials and Methods

Lyophilized cercariae of *Schistosoma mansoni* were divided into fractions of approximately 2,500 cercariae and resuspended in 50 mM  $\text{NH}_4\text{HCO}_3$ . Cells were disrupted using a Fisher 550 Sonic Dismembrator (Fisher Scientific) and spun at high speed to remove cell debris (13,500 x g, 5 minutes, 4°C, Beckman Microfuge 18). The supernatant lysate was assayed colorimetrically for protein content using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

Samples containing 400 ug of protein were digested with 200 ng proteomics grade trypsin (Sigma, St Louis, MO) in 50 mM  $\text{NH}_4\text{HCO}_3$  buffer at 37°C for 18 hours. Samples were then vacuum dried (Savant Speed Vac) and desalted using a  $\text{C}_{18}$  Omix resin tips (Varian) and mixed with matrix (alpha-cyano-4-hydroxy-cinnamic acid in 50 % acetonitrile and 0.1 % trifluoroacetic acid; Sigma, St Louis, MO) prior to deposition on a 96 well MALDI target plate (Applied Biosystems, Foster City, CA). Spectra were obtained using an Applied Biosystems Voyager DE-STR MALDI-TOF MS run in positive reflector mode ( $m/z$  500 – 5000) with 50 shots acquired per spectrum.

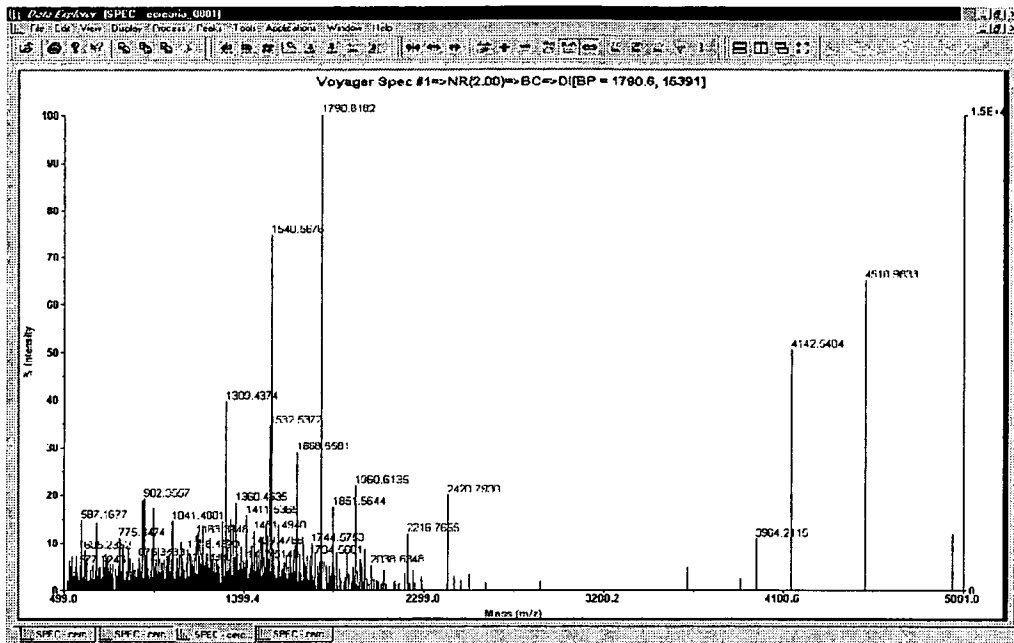
Spectra were analyzed and manipulated using Data Explorer software (Applied Biosystems). Peaks were deisotoped and an internal calibration using trypsin autolysis peaks was carried out. Mass lists were generated and compared to theoretical peptides using the NCBI databases (<http://ncbi.nih.gov>) and Mascot (Perkins et al., 1999).

## Results and Discussion

Peptide fingerprinting analysis of supernatant from disrupted cells of cercariae of *Schistosoma mansoni* using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and searching of the NCBI metazoa database yielded a stathmin-like protein of *Schistosoma mansoni* (Valle et al 1999; Perkins et al. 1999) as the best match among 170,167 sequences searched. The corresponding MALDI-TOF MS spectrum and detailed search results are provided below. The results demonstrate that infectious Schistosome cercariae can be detected successfully by MALDI-TOF MS using minimally processed crude cell extracts. This novel detection technique opens the door to the automated high-throughput analysis of environmental samples for specific Schistosome parasites. Search results indicate that the method has sufficient discriminatory power to distinguish between Schistosomes infecting birds and humans. As such, it may be used to detect the pathogen in the environment as well as in clinical specimens thereby supporting the global disease control and prevention studies conducted by the World Health Organization and other agencies (WHO 1998).

## References

- Perkins, D. N., D. J. Pappin, D. M. Creasy, and J. S. Cottrell. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**:3551-67.
- Valle, C., Festucci, A., Calogero, A., Macri, P., Mecozzi, B., Liberti, P., and D. Cioli. 1999. Stage-specific Expression of a *Schistosoma mansoni* Polypeptide Similar to the Vertebrate Regulatory Protein Stathmin\*. *J. Biol. Chem.* **274**(48):33869-33874.
- World Health Organization. 1998. Report of the WHO Informal Consultation on Schistosomiasis Control. WHO/CDS/CPC/SIP/99.2. Geneva, Switzerland. ([http://www.who.int/entity/wormcontrol/documents/publications/en/99\\_2en.pdf](http://www.who.int/entity/wormcontrol/documents/publications/en/99_2en.pdf))
- Ross, A. G., Sleight, A. C., Li, Y., Davis, G. M., Williams, G. M., Jiang, Z., Feng, Z., and D. P. McManus. 2001. Schistosomiasis in the People's Republic of China: Prospects and Challenges for the 21<sup>st</sup> Century. *Clinical Microbiol. Rev.* **14**(2):270-295.



MALDI-TOF mass spectrum of digested supernatant containing soluble proteins obtained via disruption of approximately 2,500 cercariae of *Schistosoma mansoni*. Ions detected at  $m/z$  1360.5 and 4,983.3 were matched by Mascot database searching to two peptides specific to a stathmin-like protein coded in the genome of *S. mansoni* and expressed exclusively in infectious cercariae.

Index	Centroid Mass	Lower Bound	Upper Bound	Charge (z)	Height	Relative Intensity
63	1790.63	1790.63	1790.63	0	6298	100
53	1540.624	1540.62	1540.62	0	4303	68.33
58	1668.587	1668.59	1668.59	1	2246	35.67
67	1960.602	1960.6	1960.6	0	2184	34.67
14	945.5433	945.54	945.54	1	1700	26.99
72	4983.296	4983.3	4983.3	0	1362	21.62
54	1545.577	1545.58	1545.58	0	1162	18.46
3	694.411	694.41	694.41	0	1131	17.96
37	1309.53	1309.53	1309.53	0	1126	17.88
43	1360.562	1360.56	1360.56	0	1113	17.66
9	890.522	890.52	890.52	0	1098	17.43
39	1345.506	1345.51	1345.51	1	997	15.83
2	660.4562	660.46	660.46	0	987	15.67
52	1532.605	1532.6	1532.6	1	985	15.64
65	1851.554	1851.55	1851.55	0	958	15.21
35	1294.533	1294.53	1294.53	1	923	14.65
20	1041.517	1041.52	1041.52	0	884	14.04
46	1411.599	1411.6	1411.6	1	879	13.95
10	902.4668	902.47	902.47	0	871	13.83
62	1787.702	1787.7	1787.7	0	863	13.7
23	1070.566	1070.57	1070.57	0	813	12.91
38	1333.557	1333.56	1333.56	0	784	12.45
59	1703.618	1703.62	1703.62	0	783	12.43
48	1458.555	1458.55	1458.55	0	777	12.33
47	1440.572	1440.57	1440.57	0	765	12.14
1	515.3267	515.33	515.33	0	749	11.9
60	1710.565	1710.57	1710.57	0	747	11.87
12	912.4267	912.43	912.43	0	735	11.67
51	1531.575	1531.58	1531.58	1	717	11.39
68	1982.646	1982.65	1982.65	0	716	11.37
32	1239.571	1239.57	1239.57	0	714	11.34
5	740.3522	740.35	740.35	0	688	10.93
36	1295.53	1295.53	1295.53	1	665	10.55
31	1231.445	1231.45	1231.45	0	659	10.47
61	1735.604	1735.6	1735.6	0	658	10.46
15	954.5144	954.51	954.51	0	657	10.44
27	1192.501	1192.5	1192.5	0	656	10.41
49	1468.591	1468.59	1468.59	0	648	10.29
6	819.534	819.53	819.53	0	642	10.2
4	721.4264	721.43	721.43	0	627	9.96
40	1346.541	1346.54	1346.54	1	613	9.74
55	1570.598	1570.6	1570.6	0	609	9.68
30	1217.481	1217.48	1217.48	0	608	9.65
19	1036.444	1036.44	1036.44	0	606	9.63
25	1133.523	1133.52	1133.52	0	605	9.6
33	1272.56	1272.56	1272.56	0	603	9.57

18	1017.465	1017.46	1017.46	0	601	9.54
Index	Centroid Mass	Lower Bound	Upper Bound	Charge (z)	Height	Relative Intensity
8	847.4569	847.46	847.46	0	583	9.26
13	944.5485	944.55	944.55	1	583	9.26
29	1202.521	1202.52	1202.52	0	582	9.23
28	1194.446	1194.45	1194.45	0	577	9.17
21	1044.482	1044.48	1044.48	0	577	9.16
71	2420.685	2420.69	2420.69	0	569	9.04
64	1818.496	1818.5	1818.5	0	568	9.02
69	2088.621	2088.62	2088.62	0	565	8.97
57	1667.589	1667.59	1667.59	1	563	8.94
34	1275.569	1275.57	1275.57	0	548	8.7
17	1011.458	1011.46	1011.46	0	546	8.67
50	1484.521	1484.52	1484.52	0	545	8.65
22	1068.618	1068.62	1068.62	0	533	8.46
44	1385.593	1385.59	1385.59	0	531	8.44
11	908.4091	908.41	908.41	0	524	8.33
45	1410.582	1410.58	1410.58	1	522	8.29
41	1348.52	1348.52	1348.52	1	520	8.26
24	1131.538	1131.54	1131.54	0	517	8.22
16	959.5558	959.56	959.56	0	517	8.21
7	844.48	844.48	844.48	0	515	8.17
70	2104.651	2104.65	2104.65	0	512	8.13
42	1349.51	1349.51	1349.51	1	510	8.1
66	1869.627	1869.63	1869.63	0	508	8.07
26	1162.538	1162.54	1162.54	0	507	8.06

**Table 1. List of  $m/z$  (peptides) detected by MALDI-TOF MS (Centroid mass =  $m/z$ ).**  
The sample analyzed is the same as the spectrum presented above.

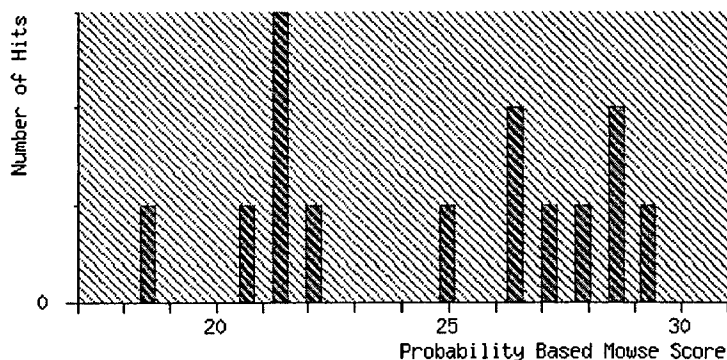
# **Mascot Search Results**

**Database** : NCBI nr 20030815 (1500836 sequences; 483636797 residues)  
**Taxonomy** : Other Metazoa (170167 sequences)  
**Timestamp** : 26 Mar 2004 at 16:29:44 GMT  
**Top Score** : 29 for gi|3641363, stathmin-like protein [Schistosoma mansoni]

## **Probability Based Mowse Score**

Score is  $-10 \cdot \log(P)$ , where P is the probability that the observed match is a random event.

Protein scores greater than 65 are significant ( $p < 0.05$ ).



## **Concise Protein Summary Report**

1.	gi 3641363	Mass: 13642	Total score: 29	Peptides matched: 2
	stathmin-like protein [Schistosoma mansoni]			
	gi 28870230	Mass: 5242	Total score: 22	Peptides matched: 1
	hypothetical protein [Pseudomonas syringae pv. tomato str. DC3000]			
	gi 22777674	Mass: 5428	Total score: 21	Peptides matched: 1
	hypothetical conserved protein [Oceanobacillus iheyensis HTE831]			
	gi 29377389	Mass: 7295	Total score: 20	Peptides matched: 1
	cold-shock domain family protein [Enterococcus faecalis V583]			
	gi 29377007	Mass: 7779	Total score: 19	Peptides matched: 1
	conserved domain protein [Enterococcus faecalis V583]			
	gi 3395365	Mass: 8018	Total score: 19	Peptides matched: 1
	non receptor serine/threonine kinase [Dugesia japonica]			

## **Search Parameters**

Type of search	: Peptide Mass Fingerprint	
Enzyme	: Trypsin	Mass values: Monoisotopic
Protein Mass	: >0	Peptide Mass Tolerance: $\pm 50$ ppm
Peptide Charge State	: 1+	Max Missed Cleavages: 3
Number of queries	: 15	

# **MASCOT** Mascot Search Results

## Protein View

Match to: **gi|3641363**; Score: **29**  
**stathmin-like protein [Schistosoma mansoni]**

Nominal mass ( $M_r$ ): **13642**; Calculated pI value: **9.02**  
NCBI BLAST search of **gi|3641363** against nr  
Unformatted sequence string for pasting into other applications

Taxonomy: Schistosoma mansoni  
Links to retrieve other entries containing this sequence from NCBI  
Entrez:  
gi|8845483 from Schistosoma mansoni  
gi|4590342 from Schistosoma mansoni

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Number of mass values searched: **15**  
Number of mass values matched: **2**  
Sequence Coverage: **47%**

Matched peptides shown in **Bold Red**

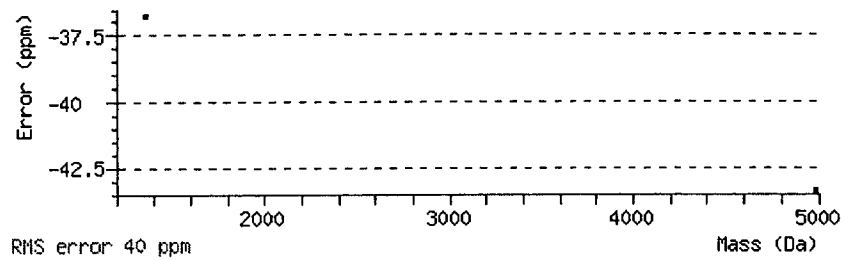
**1 MKVTPIIFAV FCVVGAMTLI TATTLEQAPH PSEKDMELVY IDAEYEKEGG**  
**51 LKSICNEIKR SFRKGRHHIY KVMDKYIRKE DLGMKMLDVA KILGRRIEKR**  
**101 MEYIAKKLDK MMEYESS**

Show predicted peptides also

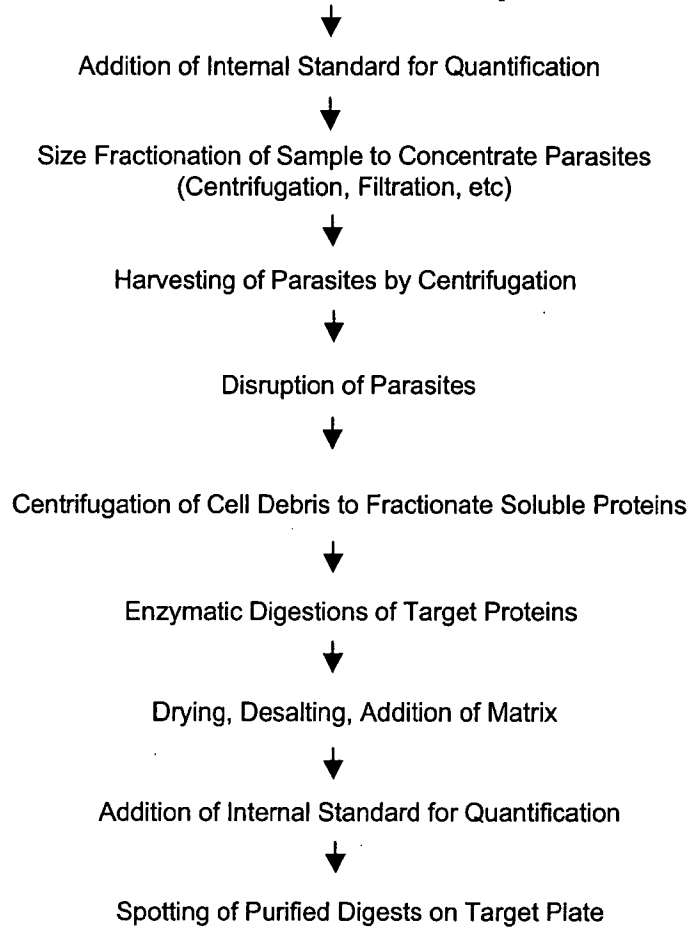
Sort Peptides By ☒ Residue Number ☒ Increasing Mass ☒ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
3 - 47	4983.30	4982.29	4982.50	-0.22	1	
						<b>VTPIIFAVFCVVGAMTLITATTLEQAPHPSEKDMELVYIDAEYEK</b>
107 - 117	1360.56	1359.55	1359.61	-0.05	2	<b>KLDKMMEYESS</b>

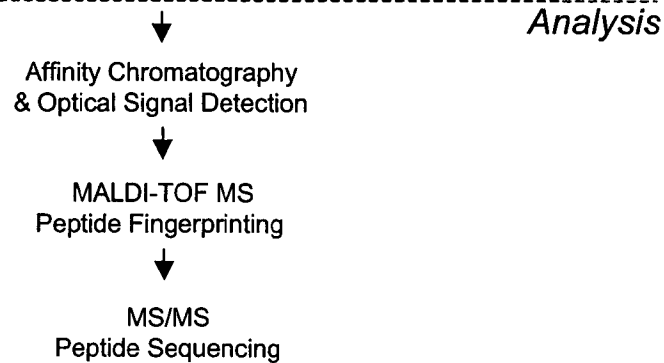
No match to: 660.46, 694.41, 890.52, 945.54, 1309.53, 1345.51, 1532.60, 1540.62, 1545.58, 1668.59, 1790.63, 1851.55, 1960.60



## Environmental / Clinical Specimen



### *Sample Preparation*



**Flow Chart Illustrating the Detection of Schistosome Parasites in Environmental and Clinical Samples. All or a Subset of Processing Steps May Be Required for Successful Identification.**